AD_____

GRANT NUMBER DAMD17-97-1-7091

TITLE: Peptide-Targeted Drug Delivery to Breast Tumors

PRINCIPAL INVESTIGATOR: Gian Paolo Dotto, M.D., Ph.D.

CONTRACTING ORGANIZATION: Massachusetts General Hospital
Boston, Massachusetts 02114

REPORT DATE: July 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 1999	Annual (1 Jul 98 - 30 Jun 99)

4. TITLE AND SUBTITLE

Peptide-Targeted Drug Delivery to Breast Tumors

5. FUNDING NUMBERS

DAMD17-97-1-7091

6. AUTHOR(S)

Dotto, Gian Paolo, M.D., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Massachusetts General Hospital
Boston, Massachusetts 02114

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Our proposal is aimed at using a genetic selection/screening technique to identify peptides that selectively recognize breast cancer cells (as opposed to normal cells of the same or different type). Such cancer-specific peptides will then be coupled to fleximer-based long-circulating drug carriers to confer upon these compounds the desired specificity. In the first year of our work, we optimized conditions and screened phage display libraries for peptides that selectively bind to Erb2, an important cell surface receptor overexpressed in breast cancer cells. Unfortunately, all peptides lost specificity of binding, when assayed independently from the phages. The challenge for the second year was to identify peptides that retain binding specificity when tested separately from the phage with which they were identified. As a more powerful method to identify Erb2-binding peptides, we have undertaken phage panning experiments with purified extracellular domain of the Erb2 protein produced in a soluble form. At the same time, we have started to produce recombinant EGF and Heregulin 1, which are well known ligands of Erb2 when paired with the EGF receptor and Erb3, respectively. The recombinant EGF and Heregulin will then be coupled to macromolecular carriers for binding to Erb2 overexpressing breast carcinoma cells, as for the experiments planned for the peptides.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer	14
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18 298-102

USAPPC V1.00

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

✓ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

✓ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

✓ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

G. Pablo Rotto 7/15/99

PI - Signature Date

Table of Contents

Introduction

Macromolecular drug carriers provide one of the most promising approaches to improve delivery of therapeutic and diagnostic drugs to cancer cells. A very significant achievement in this area relates to the recent development of "long-circulating" macromolecular and colloidal preparations (polymers, micelles, liposomes, etc.). Clearance of these compounds from the blood stream and uptake by the reticuloendothelial system (RES) are very slow. As a consequence, macromolecular drug carriers circulate long enough to extravasate into tumors via "leaky" endothelium, and accumulate at these sites as a result of deficient lymphatic drainage, non-specific tissue binding and spontaneous endocytosis.

Although long circulating drug carriers do accumulate in solid tumors, they do not specifically bind cancer cells. Thus, the microdistribution of the drug within the tumor tissue may be suboptimal. Also, the drug complex can partially return to the blood stream by a reverse of the extravasation process through the leaky endothelium, or lymphatic drainage, or both. These limitations have two major consequences: 1) therapeutic drug delivery has limited efficacy, so that the therapeutic index remains relatively low; 2) diagnostic imaging agents for tumor staging have low specificity and, for small metastases, low sensitivity. Substantial improvements could be achieved by the association of long-circulating drug carriers with "vector molecules" capable of binding specifically to cancer cells. These molecules would (a) direct carrier binding specifically to cancer cells, minimizing nonspecific uptake by the surrounding tissue; and (b) prevent the carrier from exiting the tumor, and thus maintaining higher local drug concentrations. Previous attempts to develop cancer-specific vector molecules were based mostly on antibodies against tumor-specific antigens. This approach, however, has several limitations, related to the large size of the antibody molecules, and the fact that cancer-specific antigens are usually expressed at low concentrations, and only in limited subsets of cancer cells.

Our proposal is based on the idea of using a genetic selection/screening technique to identify peptides that selectively recognize breast cancer cells (as opposed to normal cells of the same or different type). Such cancer-specific peptides will then be coupled to fleximer-based long-circulating drug carriers to confer upon these compounds the desired specificity.

Body of Work

1) Assumptions, Experimental Approach and Results.

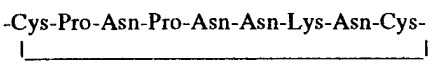
One frequent genetic alteration connected with breast cancer development is amplification and/or overexpression of the Erb2 oncogene. In order to optimize our selection/screening procedure for breast-cancer specific peptides, we decided at first to focus on the identification of peptides that can selectively bind to the extracellualr portion of the Erb2 protein. We were prompted by several reasons. First, by knowing what the specific target of the selected peptides would be, it would be possible to measure affinity of binding of the peptide-expressing phage, as well as of the biochemically synthesized peptide, either free in solution or coupled to a macromolecular carrier. This in turn would have allowed us to optimize conditions for additional studies with peptides binding to unidentified targets. Second, we made a collaborative arrangement with Dr. Kermit Carraway at Harvard Medical School, who provided us with SF9 insect cells overexpressing the Erb2 protein in a functional state on their surface. Use of normal SF9 cells for control experiments would eliminate all background problems due to binding of endogenous Erb2 which is normally expressed on the surface of mammalian cells. More recently, Dr. Carraway has provided us with the purified extracellular domain of the Erb2 protein produced in a soluble form, that we have started to use as an alternative target for our phage panning/selection assays (as described in detail further below).

Three phage display libraries have been used for these studies, that were purchased from New England Biolabs. Each of these libraries was subjecetd to several cycles of binding, saline washing, elution at acidic pH, and amplification using (a) Erb2-overexpressing versus control SF9 cells; (b) Erb2-overexpressing versus control NIH-3T3 cells; (c) Erb2 overexpressing versus non-expressing mammary carcinoma cell lines. The phage species recovered at the end of these cycles were cloned, purified and tested for selectivity of binding to Erb2 overexpressing versus control cells.

In the first year of our work we screened a library of phages expressing a random strech of 7 amino acids at the amino terminus of the phage gene III protein, and a second library with a random 12 amino acid sequence inserted at the same location. As described in detail in our previous progress report, our screening attempts yielded unsatisfactory results. We thus switched to a third library of phages that express a 7 amino acid random sequence flanked by a cystein at both ends, and inserted at the amino terminus of the gene III protein. After only two rounds of screening, more than 80% of the phages that were examined expressed the same peptide sequence. This peptide sequence was not found in 20 random phages that were examined from the initial library. The sequence of this peptide is:

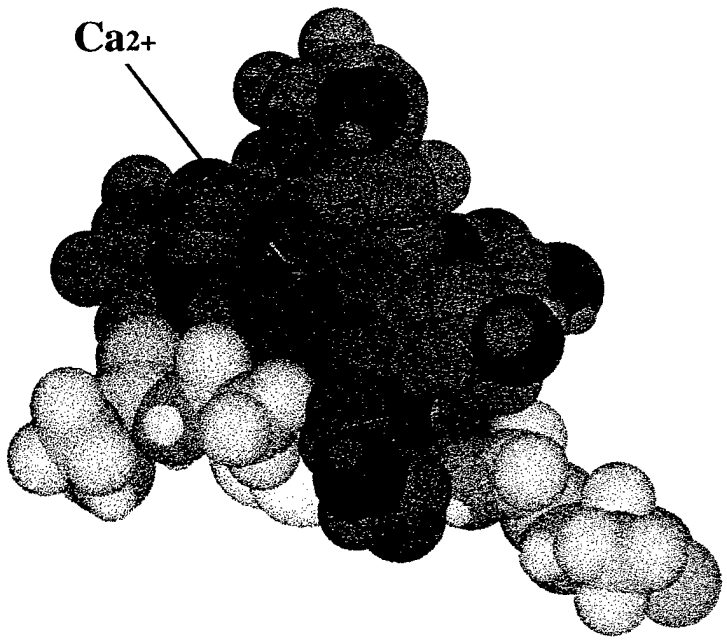
Cys-Pro-Asn-Pro-Asn-Asn-Lys-Asn-Cys

Computer simulation for the most thermodinamically-favourable configuration of the peptide pointed to a very interesting feature, namely that the peptide can be present in a cyclic form:



which can be dramatically stabilized when complexed with calcium ion (Figure 1). This finding raised the possibility that the peptide, either in its phage-bound or chemically synthesized form, binds to Erb2-expressing cells in a calcium-dependent manner. However, no such calcium-dependence was observed. Furthermore, the peptide expressed on the phage surface exhibited only a 4-7 fold increased specificity of binding on Erb2 expressing cells versus controls, and no such specificity was observed when the peptide was tested in a free soluble form or as a complex with a macromolecular carrier.

For this reason, we decided to abandon this peptide and look for an improved screening strategy that might enhance our chances of success. In particular, the main problem that we encountered in all our screening attempts, was the isolation of only a very limited number of phages expressing Erb2-binding peptides. Our priority at this point was that of isolating a much higher number of phages different expressing Erb2-binding peptides, so that we would have greater chances of identifying peptides that would maintain high affinity and specificity of binding even when tested after chemical synthesis. Thus, we devoted our subsequent efforts to the optimization of a phage panning selection using as target the purified extracellular domain of the ERb2 protein. Dr. Carraway was recently able to produce this domain of Erb2 in SF9 cells as a secreted soluble, form. The protein produced in this manner appears to retain much of its native configuration, as indicated by the fact that it is still recognized by antibodies that recognize extracellular Erb2 only under native conditions. Panning of phage libraries on the purified Erb2 instead of Erb2-expressing cells will have the significant advantage of eliminating the binding of phage to other membrane cellular proteins, and therefore should greatly increase our chances of obtaining a much larger collection of Erb2-binding phages. In our initial studies, we performed phage panning experiments with Petri dishes coated with the Erb2 protein. A blocking solution with BSA was used to prevent aspecific binding to plastic. However, these blocking conditions were too strong and need to be better optimized. Alternatively, the soluble Erb2 protein is produced as a his-tagged form, so that it will be possible to perform phage panning selection with the protein attached to beads rather than absorbed to dishes.



Steric energy (Ca-free):
Bend: 38.6981
Stretch-Bend: 0.5456
Torsion: 27.7462
Non-1,4 VDW: -52.8769
1,4 VDW: 26.9424
Dipole/Dipole: -55.9064
Total: -10.4338

Steric energy (Ca complex):
Stretch: 4.2724
Bend: 35.6513
Stretch-Bend: 0.5220
Torsion: 35.4930
Non-1,4 VDW: -28.3430
1,4 VDW: 27.9803
Charge/Dipole: -198.4313
Dipole/Dipole: -20.3061
Total: -143.1612

Figure 1. Cyclic form of Cys-Pro-Asn-Pro-Asn-Asn-Lys-Asn-Cys forms a potential metal-binding site (molecular simulation). Steric energies calculated based on MM2 model.

2) Methods : High titer (~10^{11} particles/ml) phagemid library preparations were preabsorbed in PBS to unfixed control cells, and allow to bind for 10'. Supernatant was removed and added to unfixed Erb2 overexpressing cells for 20' at room temperature. Non-bound virions were removed by repeated washes with saline solution, while binding virions were recovered with a low pH solution. After each cycle, recovered phages were amplified by growth into bacteria. The phages recovered at the end of these cycles of positive and negative selection were cloned, purified and retested for their specificity of binding to Erb2 expressing versus control cells, and the sequence of their random amino acid inserts was established. Similar panning experiments were also repeated with petri dishes to which the purified extracellular domain of the Erb2 protein was previously absorbed (using a protein concentration of 0.1mg/ml, in PBS). After coating with the Erb2 proteins, dishes were treated with a "blocking solution" with 0.1 mg/ml BSA, to prevent the aspecific binding of phage to plastic. Unfortunately, we found that under these conditions a significant number of phage bound aspecifically to BSA, so that no specific Erb2-binding phages could be recovered.

The complementary part of this work involved the synthesis and purification of a peptide analogous to the specific random peptide expressed by the Erb2-binding phages. The purified peptide was labeled with a radioactive or fluorescence moiety and tested for its specificity of binding to Erb2 overexpressing versus control cells. The same peptide was also coupled to a macromolecular drug carrier. The peptide comprising a terminal cystein (incorporated during peptide synthesis) was conjugated with S-thiopyridyl groups of the carrier. The latter were introduced into the carrier

structure via carrier conjugation with cystamine, with subsequent reduction of the S-S bridge followed by the thiol group reaction with dithiopyridine. The peptide coupled to the macromolecular carrier was tested on Erb2 overexpressing versus control cells as before.

3) Model carriers for drug targeting with cooperative vector molecules.

While testing phage display libraries for breast cancer-targeted peptides, we continued developing prototype cooperative carrier systems utilizing a previously developed model system based on N-formylpeptide - formylpeptide receptor interaction.
Peptides binding membrane markers of white blood cells, including the leukocyte N-formylpeptide receptor (FPR), have been found to accumulate in inflammatory regions as a result of association with white blood cells invading the inflammation site. This fact was utilized to study the behavior of cooperative polymer-based vectors comprising several peptide moieties, as compared to the analogous monomeric peptide. The objectives of this fragment of the study were: (i) prepare macromolecular preparations of varying length and comprising varying number of chemotactic peptide (N-formyl-Met-Leu-Phe-Lys) moieties per macromolecule length; (ii) determine the optimal range of peptide content; (iii) determine the molecule size range providing a most favorable blood clearance rate for agent accumulation in inflammatory areas; (iv) synthesize a model agent for inflammation scintigraphy comprising a g-emitting radionuclide and a fluorescent label; (v) characterize the biokinetics and imaging characteristics of the model preparation as compared to the radiolabeled peptide monomer.

Macromolecules comprising multiple f-Met-Leu-Phe-Lys (fMLFK) moieties and DTPA were synthesized using linear poly[hydroxymethyl-ethylene hydroxy-methyl-formal] (PHF) backbone. PHF, a biomimetic "stealth" polymer, is a base compound of Fleximer family. An aldehyde form of PHF with broad molecular weight distribution (10 to 150 kDa) was conjugated with cystamine (spacer precursor) and fMLFK in one stage, at various molar ratios, in the presence of cyanoborohydride. The conjugates were subsequently reduced with borohydride to transform the unused aldehyde into hydroxymethyl groups and S-S bridges to mercaptogroups. The latter were derivatized with DTPA anhydride and (in some preparations) with trace amounts of fluorescein-5-maleimide. The resultant polymers were purified by gel chromatography, fractionated by SEC HPLC, and labeled with 111In via transchelation in citrate buffer. Control polymers containing DTPA but no peptide were prepared analogously. Radiolabeled peptide monomer was prepared via fMLFK derivatization with DTPA and labeling with 111In.

Pilot biokinetics and biodistribution studies were performed in normal male CD rats (n=3 per preparation) to determine the optimal ranges of peptide content and molecular weight of the polymer. Conjugates with broad molecular weight distribution were fractionated by SEC HPLC to obtain fractions with MW=10, 50 and 80 kDa (MW distributions overlapped by ca. 30% by HPLC). For the preliminary testing, conjugates containing from 0.5 to 20 % fMLFK (mol/mol monomer) were prepared. Pilot studies showed that the optimal range by conjugate content is ca. 3-10%, and the optimal dose range, with regard to clearance rate, is > 0. 1 mg/kg.

For the biodistribution studies in rabbit inflammation model, preparations containing 5% of each fMLFK and DTPA were synthesized. Non-overlapping 15 and 75 kDa fractions used in this experiment showed statistically significant difference in biokinetics. The preparations were labeled with 111In and injected iv into 2.5 kg New Zealand rabbits, n=4 per group, 50-100 μCi/animal (0.5 mg/kg total substance). Animals were normal or bearing focal bacterial inflammation induced by inoculation of E.Coli (clinical isolate) in thigh muscle. Indium-labeled PHF-DTPA and monomeric DTPA-fMLFK were used as control preparations. Images were acquired over a 20 hr. period, followed by a biodistribution study. Biodistribution data showed that, compared to the monomer DTPA-fMLFK, renal accumulation was greatly reduced (by 81% and 88% for 70 and 15 kDa preparations respectively), while accumulation in the infected muscle was reduced by only 37% and 72%. Target to normal muscle ratios were 25±10 and 14±4, compared to 33±21 for the monomer. In blood, testes, adrenals and heart, accumulation was reduced by 40-50%. Hepatic and splenic depositions were reduced by 40% for the 15 kDa preparation, and increased by 50% for the 70 kDa one. In other tissues, label content did not significantly differ from the monomer. By 20 hr., accumulation of DTPA-PHF-fMLFK in the infected site resulted in clear delineation of the inflammation in all images. It has been found that the high molecular weight preparation accumulate in the inflammation, in part, as a result of non-specific vascular leakage/retention, analogous to that in tumors. Accumulation of the low molecular weight preparation in the inflammatory site was completely target-specific, i.e., entirely dependent on the action of the chemotactic peptide. Based on this observation, we hypothesize that small targeted polymers may be preferable as targeted carriers; this hypothesis will be further tested in the ongoing research.

Conclusions

Long circulating drug carriers accumulate in solid tumors, but do not specifically bind cancer cells. Thus, therapeutic drug delivery has limited efficacy and diagnostic imaging agents have relatively low specificity and, for small metastases, low sensitivity. Substantial improvements could be achieved by the association of long-circulating drug carriers with "vector molecules" capable of binding specifically to cancer cells. Our proposal is based on the idea of using a genetic selection/screening technique to identify peptides that selectively recognize breast cancer cells (as opposed to normal cells of the same or different type). Such cancer-specific peptides will then be coupled to fleximer-based long-circulating drug carriers to confer upon these compounds the desired specificity. In the first year of our work, we optimized conditions and screened three different phage display libraries for peptides that selectively bind to Erb2, an important cell surface receptor overexpressed in breast cancer cells. We confirmed that the phage display technology can be used to identify peptides that bind to proteins expressed on the surface of breast cancer cells. The challenge for the second year of our work was to identify peptides that retain specificity of binding when tested separatedly from the phage with which they were identified. Unfortunately, all peptides lost specificity of binding, when assayed independently from the phages, either in a free soluble formand/or coupled to a macromolecular carrier. As an alternative method to identify Erb2-binding peptides with higher selectivity of binding, we have undertaken phage panning experiments with the purified extracellular domain of the Erb2 protein produced in a soluble form. Work for the upcoming year will be aimed at optimizing our panning conditions for the purified Erb2, and for the selection of phages with high affinity and specificity of binding. At the same time, we will be producing large amounts of recombinant EGF and Heregulin 1, which are well known ligands of Erb2 when paired with the EGF receptor and Erb3, respectively. The recombinant EGF and Heregulin will then be coupled to macromolecular carriers for binding to Erb2 overexpressing breast carcinoma cells, as for the experiments that were originally designed for the Erb2-specific peptides.

References

Gallop, MA, Barrett, RW, Dower, WJ, Fodor, SPA, and Gordon, EM (1994). Applications of combinatorial technologies to drug discovery. 1. Background and peptide combinatorila libraries. J. Med. Chem., 37, 1233-1251.

Cassidy, J., Newell, D.R., Wedge, S.R., and Cummings, J. (1993) Pharmacokinetics of high molecular weight agents. Cancer Surveys, 17, 315-341.

Kotani, M., Matsuno, K., Ezaki, T., and Ueda, M. (1980). The increase in permeability of postcapillary venules in lymph nodes subjected to the regional graft-versus-host reaction. Lab. Investigation 42, 589-595

Frank, M.M., and Fries, L.F. The role of complement in inflammation and phagocytosis (1991). Immunology today, 12, 322-326.

Royall, J.A., Berkow, R.L., Beckman, J.S., Cunningham, M.K., Matalon, S., and Freemen, B.A. (1989) Tumor necrosis factor and interleukin 1 alpha increase vascular endothelial permeability. Am. J. Physiol. 257 L399-L410.

Fujita, S, Puri, R., Yu, Z.-X., Travis, W., Ferrans, V. (1991) An ultrastructural study of in vivo interactions between lymphocytes and endothelial cells in the pathogenesis of the vascular leak syndrome induced by interleukin-2. Cancer, 68, 2169-2174.

Jain RK. Transport of molecules across tumor vasculature. Cancer & Metastasis Reviews. 6(4):559-93, 1987.

Gregoriadis, G. (Ed) Liposome Technology, 2nd Edn., CRC Press, Boca Raton, 1993.

Woodle MC. Sterically stabilized liposome therapeutics. Adv. Drug Delivery Rev. 1995, 16:249-266

Gref R, Domb A, Quelec P, Blunk P, Muller RH, Verbavatz JM, Langer R. The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanospheres. Adv. Drug Delivery Rev. 1995, 16:215-234

Papisov MI. Modeling in vivo transfer of long-circulating polymers (two classes of long circulating polymers and factors affecting their transfer in vivo). Adv. Drug Delivery Rev., Special issue on long circulating drugs and drug carriers, 1995, 16:127-137.

Papisov MI, Weissleder R, Brady TJ. Diagnostic targeting of lymph nodes with polymeric imaging agents. In: Torchilin VP, ed. Targeted delivery of imaging agents. CRC Press, Boca Raton, 1995: 385-402

Papisov MI, Weissleder R, Schaffer B, Bogdanov AA, Brady TJ. Intravenous

carriers for drug delivery to lymph nodes. In: Sixth International Symposium on Recent Advances in Drug Delivery Systems, Salt Lake City, UT. University of Utah, 1993; 137-138.

Papisov MI, Garrido L, Poss K, Wright C, Weissleder R, Brady TJ. A long-circulating polymer with hydrolyzable main chain. In: 23 International Symposium on Controlled Release of Bioactive Materials, Kyoto 1996, CRS 1996, in press.

Papisov MI, Poss K., Weissleder R., and Brady TJ. Effect of steric protection in lymphotropic graft copolymers. In: Seventh international symposium on recent advances in drug delivery systems, Abstracts; Salt Lake City, UT, 1995; University of Utah 1995, 171-172

Papisov MI, Martin C, Poss K., Weissleder R., and Brady TJ. Amplification of steric effects in cooperative systems. In: 22-nd International Symposium on Controlled Release of Bioactive Materials, 1995, 442-443.

Torchilin VP, Omelyanenko V, Papisov MI, Bogdanov AA, Trubetskoy VS, Herron DIN, Gentry CA. Poly(ethyleneglycol) on the liposome surface: on the mechanism of polymer-coated liposome longevity. BBA 1994, 1195:11-20.

Poss K, Bogdanova AV, Weissleder R., Brady TJ, Papisov MI. Cytotoxicity of doxorubicin covalently linked with the backbone of lymphotropic graft copolymers. In: Seventh international symposium on recent advances in drug delivery systems, Abstracts; Salt Lake City, UT, 1995; University of Utah 1995, 173-174

Harika L, Weissleder R, Poss K, Papisov M. A macromolecular intravenous agent for MR lymphography: characterization and efficacy studies. Radiology 198:365-370, 1996

Papisov MI, Savelyev VY, Sergienko VB, Torchilin VP. Magnetic drug targeting (I) In vivo kinetics of radiolabeled magnetic drug carriers. Int J Pharm 1987; 40:201-6.

Goodson, RJ, Doyle, MV, Kaufman, SE, and Rosenberg, S (1994). High-affinity urokinase receptor antagonists identified with bacteriophage peptide display. Proc. Natl. Acad. Sci. USA 91, 7129-7133.

Yayon, A., Aviezer, D., Safran, M., Gross, J.L., Heldman, Y., Cabilly, S., Givol, D. and Katchalski-Katzir, E. (1993). Isolation of peptides that inhibit binding of basic fibroblast growth factor to its receptor from a random phage-epitope library. Proc. Natl. Acad. Sci. USA 90, 10643-10647.

Ealovega, M.W., McGinnis, P.K., Sumantran, V.N., Clarke, M.F. and Wicha, M.S. (1996). bcl-x_S gene therapy apoptosis of human mammary tumors in nude mice[1]. Cancer Research 56, 1965-1969.

Fink, LM and Hsu, P-L (1994). Bacteriophage epitope libraries. Virchows Archiv. 424, 1-6.

Smith, G.P. and Scott, J.K. (1993) by Academic Press Inc. Libraries of peptides and proteins displayed on filamentous phage. Meth. Enzy. 217, 228-257.

Koivunen, E., Wang, B., Dickinson, C.D. and Ruoslahti, E. (1994) by Academic Press Inc. Peptides in cell adhesion research. Meth Enzy. 245, 346-369.

Folgori, A., Tafi, R., Meola, A., Felici, F., Galfre, G., Cortese, R., Monaci, P. and Nicosia, A. (1994). A general strategy to identify mimotopes of pathological antigens using only random peptide libraries and human sera. EMBO J. 13, 2236-2243.

Yayon, A., Aviezer, D., Safran, M., Gross, J.L., Heldman, Y., Cabilly, S., Givol, D. and Katchalski-Katzir, E. (1993). Isolation of peptides that inhibit binding of basic fibroblast growth factor to its receptor from a random phage-epitope library. Proc. Natl. Acad. Sci. USA 90, 10643-10647.

Pascualini, R. and Ruoslahti, E. (1996). Organ Targeting in vivo using phage display peptide libraries. Nature, 380:364-366